Chemical Variability of the Volatile Metabolites from the Caribbean Corals of the Genus *Gorgonia*

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Gorgonia, Volatile Metabolites, Terpenes

The chemical composition of the investigated gorgonians showed a high level of individual variation and the colonies, according to their major contributors, were assigned to 10 distinct chemical profiles, among which A, C, E, and G were the most abundant ones. From the metabolites identified in the present study, either by means of GC/MS or using NMR techniques after conventional separation procedures, the novel cyclic ether 5,10-epoxymuurolane is found in significant quantities in D and I chemical profiles. Furanotriene, isofuranotriene and furanodiene could be referred as the most common metabolites of the genus, since they are found in 6 out of 10 chemical profiles. Isosericenine is, also, a significant contributor of H and I chemical profiles. A number of sesquiterpene hydrocarbons, such as curzerene, bicyclogermacrene, valencene, β-bourbonene and β-elemene, along with the oxygenated sesquiterpenes elemanolide and furoventalene, are present at varying concentrations in the majority of the chemical profiles. Metabolites of high discriminant value are: α-himachalene for the K chemical profile, α-santalene and its oxygenated derivatives for the G chemical profile and the three geometrical isomers of germacrone for the F chemical profile.

Several chemical profiles showed narrow geographic distribution. Most of the chemical profiles are located in the north, while F inhabits mainly southern sites and the others are equally distributed. Finally, 91% of the chemical profiles of the gorgonian colonies appeared to grow in all depths, while 9% did not inhabit deep-water environments. Most chemical profiles are less frequent at higher water depths with the exception of chemical profiles A and C.

Introduction

In the framework of our chemical and biological investigations on the volatile metabolites of marine and terrestrial organisms (Mihopoulos *et al.*, 1999; Roussis *et al.*, 1999a; Roussis *et al.*, 1999b), we were recently able to collect and study several specimens of the genus *Gorgonia*.

The octocoral fauna of the West Indies is unique in its profusion and conspicuousness of gorgonian corals. Extensions of the West Indian region reach into the Gulf of Mexico, all the Antilles, the Bahamas, the Florida Keys, the Bermudas, the Islands of the Caribbean and south along the Northeast coast of South America to the reefs of Brazil. Gorgonian octocorals represent, as estimated, 38% of the known fauna (Bayer, 1961; Cairns, 1977). Six species have been documented to be found along the West Indian Region (Rodriguez, 1995). However, within the limits of the Bahamian islands may occur only three: *Gorgonia ventalina, G. flabellum, and G. mariae* (Human, 1994).

Despite the abundant distribution of this interesting group of colonial marine invertebrates, only a few reports providing new insights into the systematics, ecology and evolution of these organisms, have appeared (Preston, 1975; Paul, 1994).

Chemical systematics of several Gorgonaceae members established on terpenoid profiles have facilitated evolution studies of these organisms (Coll, 1992). Nineteen species of gorgonians, analysed in previous projects, produced cladograms that agree for the most part with classical systematics yielding in addition several new insights into

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the evolution and classification of gorgonians. Each of the investigated species appeared to possess its own specific set of secondary metabolites (Gerhart, 1983). Several secondary metabolites have been isolated from various gorgonian extracts, mainly marine steroids of the gorgosterol type, sesquiterpene hydrocarbons and oxygenated sesquiterpenes (Cambie, 1988). Several benzofuran type derivatives, such as furoventalene, along with germacrenes, furanogermacrenes and elemanolides have been reported as widely distributed within the family Gorgoniidae. From the genera *Pacifigorgia*, *Muricea* and *Pseudopterogorgia*, for example, the structures of germacrene diol, (+)-germacrene-D (Izac *et al.*, 1982), furanodiene, sericenine, isosericenine, furanotriene, isofuranotriene and elemanolide, have been previously isolated and identified spectroscopically (Chan *et al.*, 1990).

The aim of the present study was to investigate the chemical composition of the Caribbean gorgonians, to determine the structures of the volatile secondary metabolites and evaluate their potential as a chemotaxonomic tool. Preliminary results on the chemical variability of the genus *Gorgonia* have appeared in earlier reports (Roussis *et al.*, 1995). For the needs of this project, a total of 292 specimens were collected from the Bahamian islands in the Caribbean Sea. The collected specimens, after their removal from the water, revealed a wide range of very distinct and easily observable odors. These «aroma»-variations suggested chemical variability among the gorgonian taxa and individuals.

The essential oil composition of the specimens was determined by means of GC/MS. The contributions of the individual metabolites were calculated based on the peak integrals. The majority of the metabolites were identified by combination of GC/MS and NMR methods.

Experimental

Specimen collection and vouchers

Gorgonia specimens were hand picked by scuba divers on board the R/V Seward Johnson, during the 1995 Scripps Institution of Oceanography expedition in the Caribbean Sea. Colonies of the same maturity stage were collected at different water depths from 9 geographic localities extending from the north to the south Bahamian islands. The colonies were separated immediately after their collection and placed in plastic bags that were stored at −20 °C during the cruise. The collection was shipped to Greece in dry ice (−78 °C) and were kept in deep freeze until they were analysed.

Voucher specimens are kept at the Laboratory of Pharmacognosy Herbarium, University of Athens (ATPH/MO/39–48).

Chemical analyses

The gorgonian colonies were separately hydrodistilled for 3 hrs in a Clevenger modified apparatus with a water-cooled oil receiver, to reduce hydrodistillation-overheating artifacts.

Because the harsh conditions of steam distillation have in the past been suspected as a source of artifacts, the head space volatiles of a number of gorgonian specimens were also, collected and analysed. The chemical profiles obtained by both techniques were qualitatively identical and the study was safely carried out using the essential oils.

GC-MS analysis

The GC analyses were performed on a Perkin Elmer 8500 gas chromatograph equipped with an on column injector (200 °C) and a flame ionisation detector (250 °C). He (2 ml/min) was used as carrier gas and the capillary column used was DB-5 (30m × 0.32 mm × 0.25 μm film thickness). The initial temperature of the column was 60 °C and then 280 °C with a 3 °C/min rate. Mass spectra were obtained from a Hewlett Packard 5973–6890 GC-MS system operating on EI mode (equipped with a HP 5MS 30m × 0.25 mm × 0.25 μm film thickness capillary column). The thermal program was the same as that used for the GC analyses. Identification of the chemical constituents was based on comparison of the retention time (RT) values and mass spectra with those obtained from authentic standards and/or the NIST/NBS and Wiley library spectra. The GC peak areas, without corrections for response factors were used for the quantification of the oil components.

Chromatographic separations

The organic extracts (CH_2Cl_2 / MeOH) of several gorgonians were chromatographed on silica gel with mixtures of EtOAc in cyclohexane. The non polar fractions containing the constituents of interest were further purified on normal phase HPLC (Pharmacia), on a Spherisord OD column, with cyclohexane/EtOAc as mobile phase, using a UV (LKB 2141) or refractive index (GBC LC 1240) detector.

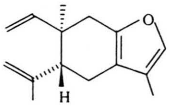
Statistical analysis

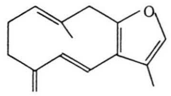
Throughout this study only chemical profile averages are shown though original data of terpene profiles of individual gorgonians are actually analysed. In this report, cluster analysis of standardised terpene data was employed. The linkage strategy was the Ward's variance minimising method and the distance was Euclidean distance (Orloci and Kenkel, 1985).

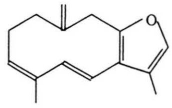
Results and Discussion

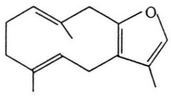
The essential oils of the 292 specimens collected in the Bahamas Region, from various geographic locations and depths, were analyzed.

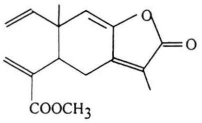
CHEMICAL PROFILE A


curzerene


furanotriene

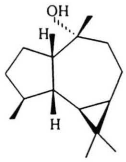

isofuranotriene


furanodiene


elemanolide

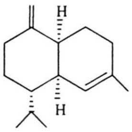
CHEMICAL PROFILE B

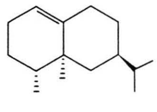
Curzerene
Ledol
Furanotriene
Isofuranotriene
Furanodiene
Elemanolide

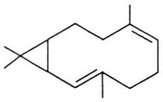

ledol

CHEMICAL PROFILE C

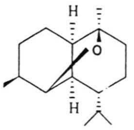
γ-Muurolene
Valencene
Bicyclogermacrene
Curzerene
Furanotriene
Isofuranotriene
Furanodiene
Elemanolide


γ-muurolene


valencene


bicyclogermacrene

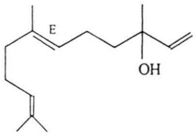
CHEMICAL PROFILE D



5,10-epoxymuurolane

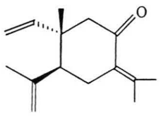
CHEMICAL PROFILE E

Furanotriene
Isofuranotriene
Unknown M^+ 230
E-Nerolidol

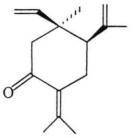


E-nerolidol

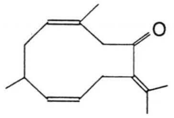
CHEMICAL PROFILE F



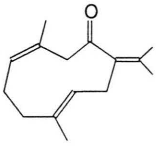
trans-β-elemenone



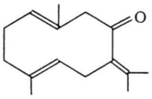
cis-β-elemenone



cis,cis-germacrone



cis-C3-C4,*trans*-C7-C8-germacrone



germacrone

More than 50 volatile compounds have been identified. The volatile constituents of the colonies were subsequently processed, as described above, and submitted to principal component analysis that led to the recognition of 10 distinct chemical varieties (chemical profiles) (Orloci and Kenkel, 1985). From the 10, A-K, chemical profiles, the A, C, E, and G were found to be the most abundant ones. Statistical analysis showed that despite their chemical similarity, profiles A-C were significally distinct. The results are reported in Table I.

The chemical profiles are defined upon the statistical processing of the chemical composition of all detected and quantified metabolites (Fig. 1). The A, B, C and E chemical profiles contain furanotriene, isofuranotriene and furanodiene as the major components. The B chemical profile is further distinguished by the presence of significant amounts of ledol (23%), while chemical profile C contains a group of sesquiterpene hydrocarbons, such as curzerene, bicyclogermacrene, valencene, β-bourbonene and β-elemene, in considerable amounts. The F chemical profile is exclusively characterised by the three geometrical isomers of germacrone in high amounts, while α-santalene and its oxygenated derivatives 12,13-epoxy-α-santalene and dihydro-α-santalen-12-one, are representative of the G chemical profile. The H and I

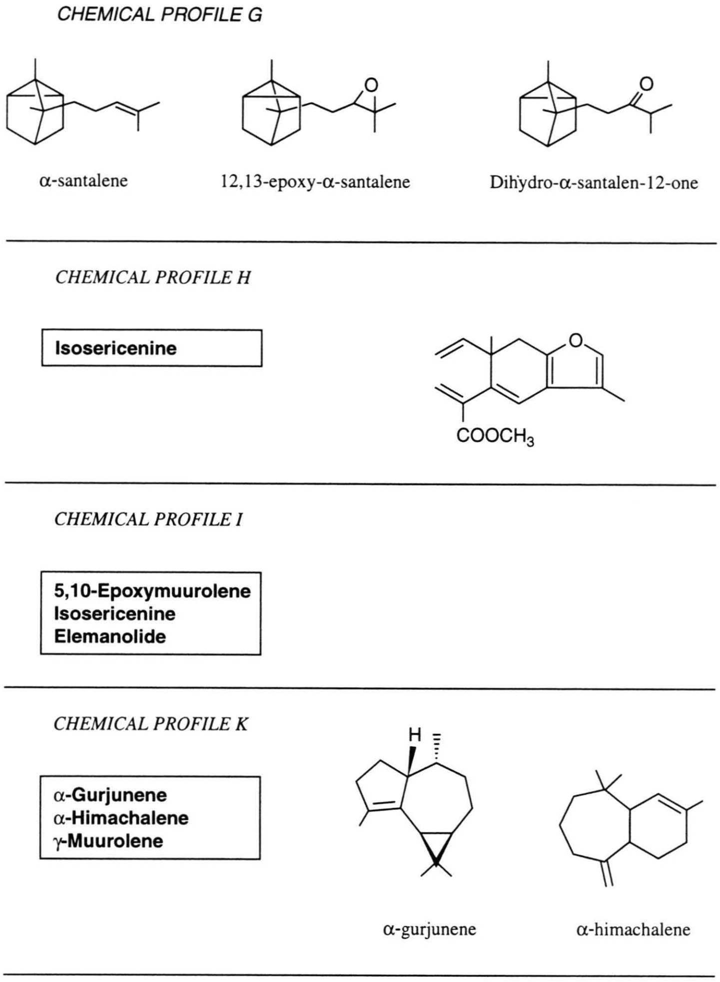


Fig. 1. Characteristic components of the chemical profiles assigned to the genus *Georgia.*

chemical profiles contain isoserisenine in high amounts (83% and 17%, respectively). The I chemical profile is further differentiated by the presence of 5,10-epoxymuurolane (40%), which is also a major component of the D chemical profile (89%). Finally, the K chemical profile is characterised by the presence of α-himachalene in 67%.

The oxygenated cyclic sesquiterpene 5,10-epoxymuurolane is a novel compound, found in the genus *Gorgonia* (Tsitsimpikou *et al.*, 1999). Only two other cyclic ethers, other than epoxides, the 1,4-epoxycadinane and 4,10-epoxymuurolene, have also been isolated from marine organisms (Amico *et al.,* 1978; Rogers and Keay, 1989). The furanosesquiterpenes, furanotriene, isofuranotriene and furanodiene are frequently found within gorgonian octocorals. Furanotriene was originally reported from the coral *Pseudopterogorgia americana* (Izac *et al.*, 1982), while, a few years later the structural elucidation of isofuranotriene from the same organism was published (Chan *et al.*, 1990). *trans, trans*-Germacrone, one of the major constituents of the F chemical profile has been isolated previously from the Indian and African medicinal plant *Fagara chalybea* (Hostettmann *et al.*, 1977). The other two geometric isomers, *cis,cis*-germacrone and *cis*-C3,C4-*trans*-C7,C8-germacrone have been chemically synthesized (Takeda *et al.,* 1973). 12,13-Epoxy-α-santalene, found exclusively in chemical profile G, had been isolated pre-

Table I. Percentage contribution of the identified volatile metabolites in the 10 recognised chemical profiles of the genus *Gorgonia*.

Substance	Identification mode	Chemical profile									
		A	B	C	D	E	F	G	H	I	K
		% Essential oil composition									
(*E,E*)-2,4-Decadienal	GC/MS	0.5 (0.02)*	0.2 (0.02)								
δ-Elemene GC/MS	0.2 (0.01)	0.1 (0.03)	0.5 (0.01)			0.2 (0.03)			0.1 (0.01)		
β-Bourbonene	GC/MS		0.4 (0.04)	3.8 (0.14)			0.2 (0.01)			0.2 (0.02)	
β-Elemene	GC/MS	0.2 (0.02)	0.2 (0.01)	2.4 (0.08)			0.1 (0.01)			0.2 (0.01)	
Longifolene	GC/MS									0.3 (0.01)	
Cyperene	GC/MS		3.1 (0.42)								
α-Gurjunene#	GC/MS		2.4 (0.25)								**5.5** (0.25)
α-Santalene	NMR						0.4 (0.09)	**5.2** (0.66)			
β-Cedrene	GC/MS		0.2 (0.01)	1.1 (0.03)							
β-Selinene	GC/MS					1.0 (0,03)					
β-Gurjunene	GC/MS	1.2 (0.11)	0.2 (0.02)	1.0 (0.03)							
α-Himachalene	GC/MS		0.6 (0.03)	1.5 (0.05)							**66.8** (4.06)
γ-Cadinene	GC/MS					0.6 (0.01)					
α-Alaskene	GC/MS					0.8 (0.03)					
Lilial	GC/MS					2.9 (0.08)					
Seychellene	GC/MS		0.1 (0.01)							1.6 (0.04)	
α-Patchoulene	GC/MS		0.6 (0.03)								2.2 (0.03)
cis-Muurola-4(14),5-diene	GC/MS			0.6 (0.03)						0.6 (0.07)	

Table I (continued).

Substance	Identification mode	Chemical profile									
		A	B	C	D	E	F	G	H	I	K
		% Essential oil composition									
Germacrene D	GC/MS		0.8 (0.04)								
γ-Muurolene	GC/MS		1.2 (0.08)	**6.9** (0.35)							11.6 (0.92)
Valencene	**GC/MS**			**7.5** (0.52)							
Viridiflorene	GC/MS										1.7 (0.08)
Bicyclogermacrene	GC/MS			**5.3** (0.18)							
Curzerene	GC/MS	**5.6** (0.18)	**4.3** (0.15)	**8.9** (0.29)						1.1 (0.08)	
β-Curcumene	GC/MS							1.7 (0.75)			
δ-Cadinene	GC/MS										0.3 (0.03)
$\Delta^{13,14}$-Iso-α-Santalol	NMR							**9.0** (1.38)			
12, 13-Epoxy-α-santalene	NMR							**68.3** (7.86)			
Selina-3,7(11)-diene	GC/MS				1.4 (0.91)						
Germacrene B	GC/MS	1.0 (0.07)		0.9 (0.04)			0.7 (0.02)				
Dihydro-α-santalen-12-one	NMR							**12.8** (1.72)			
5,10-Epoxymuurolane	NMR				**89.4** (4.52)					**44.3** (2.81)	
Ledol	GC/MS		**22.9** (1.72)		1.5 (0.09)				3.9 (0.81)	1.2 (0.07)	0.3 (0.04)
Caryophyllene oxide								2.1 (0.98)			
Furanotriene	NMR	**48.3** (2.31)	**28.4** (1.83)	**25.2** (1.45)	2.8 (0.15)	**30.6** (0.18)					1.8 (0.05)
***cis*-β-Elemenone**	NMR						**10.8** (0.92)				

Table I (continued).

Substance	Identification mode	Chemical profile									
		A	B	C	D	E	F	G	H	I	K
		% Essential oil composition									
β-Himachalene oxide	GC/MS			0.7 (0.09)							
***trans*-β-Elemenone**	NMR						**3.3** (0.28)				
Cedr-8,15-en-9α-ol	GC/MS										0.9 (0.06)
10-Epi-γ-eudesmol	GC/MS									1.8 (0.01)	
Isofuranotriene	NMR	**11.8** (0.18)	**12.1** (0.18)	**14.1** (0.18)		**2.7** (0.04)				0.1 (0.01)	
Furanodiene	NMR	**3.3** (0.05)	**2.8** (0.03)	**3.4** (0.15)			3.7 (0.81)				0.2 (0.01)
Atractylone	GC/MS	0.6 (0.03)		1.1 (0.01)						0.2 (0.02)	
***cis*-C3-C4, *trans*-C7-C8-Germacrone**	NMR						**46.8** (5.42)				
***cis, cis*-Germacrone**	NMR						**12.2** (2.17)				
***trans, trans*-Germacrone**	NMR						**17.2** (2.13)				
Unknown W=230						5.2 (0.25)					
Unknown W=230						**18.0** (0.83)					
Phenol-2-1,2-dimethyl-2-cyclopentyl)-etate	GC/MS	2.1 (0.11)									
***E*-Nerolidol**	NMR					**8.3** (0.71)					
Furoventalene	NMR	2.3 (0.14)	0.6 (0.03)	1.0 (0.02)		1.7 (0.11)				0.1 (0.01)	
Guaiol acetate	GC/MS									1.1 (0.06)	
γ-Eudesmol acetate	GC/MS			1.2 (0.06)							
α-Eudesmol acetate	GC/MS				0.5 (0.11)						

Table I (continued).

Substance	Identification mode	A	B	C	D	E	F	G	H	I	K
		% Essential oil composition									
2-α-*trans*-Bergamotol acetate	GC/MS										0.2 (0.02)
Isosericenine	NMR								**83.6** (8.67)	**32.7** (4.32)	
Elemanolide	NMR	**4.9** (0.35)	2.2 (0.11)	1.5 (0.08)		2.7 (0.11)			3.4 (0.81)	3.2 (0.45)	
Sericenine	NMR								3.5 (0.67)	1.1 (0.03)	
Manool	GC/MS	1.3 (0.28)		0.5 (0.03)							
Identified metabolites as % of total metabolites		83.3	83.4	89.1	95.6	74.5	95.6	99.1	94.4	89.9	91.5

* The numbers in parentheses represent the standard deviations.

\# Components and numbers in bold characters mark the major components of the essential oils.

viously and identified from the terrestrial root-bark of *Severina buxifolia* along with the other α-santalene derivatives found in the G chemical profile (Wu *et al.*, 1984). Sericenine and isoserisenine isolated previously from two species of the *Muricea* genus (Tursch *et al.*, 1978) are also common in the genus *Gorgonia*. Elemanolide has been isolated from a *Pseudopterogorgia* species (Chan *et al.,* 1990). β- and δ-Elemene, have also been found in the genus *Eunicea* (Tursch *et al.*, 1978) and furoventalene, found in the chemical profiles A, B, C, E and I, has been reported as a *G. ventalina* metabolite (Weinheimer *et al.*, 1969). Germacrene D, a minor constituent of the B chemical profile, occurs both in marine and in terrestrial organisms such as gorgonians, pine trees, etc (Roussis *et al.*, 1994) and, finally, germacrene B, has been also found in citrus trees (Mondello *et al.*, 1997).

In an attempt to correlate the chemical composition with the morphological characters, all specimens were initially recognised, on the basis of the conventional taxonomic criteria, either as *G. ventalina* (78 colonies), as *G. flabellum* (197 colonies) or as *G. mariae* (7 colonies). The comparison showed that no direct correlation between the chemical profiles and the typified taxa could be attributed. In fact, 9 of the 10 chemical profiles were represented by samples of all three species, and only profile D, which occurred in just 11 of the 292 samples examined, was unique to one species, *G. flabellum*. Chemical profile D however occurred in just 11 of the 197 colonies of *G. flabellum* examined. The collected colonies exhibited a noticeable variation in colour varying from brown, purple-brown, purple, brown-grey, grey, grey-yellow to yellow.

The geographic position of the collection sites was, also, correlated with the chemical profiles. The collection started from the Northern islands of the Bahamas and ended at one of the Southern tips of the same area, as shown in Table II. The A, B and C chemical profiles were found to be the most abundant at Sweetings Cay, which is located at the northern end of the Bahamas region. In the Southern sites the most prevailing chemical pro-

Table II. Geographical variation of the studied gorgonian soft corals.

	CHEMICAL PROFILES									
COLLECTION	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**K**
SITES*	% Observed Frequency[1]									
Sweetings Cay	**67**	**45**	**38**	9	**22**	21	20	0	0	0
Eleuthera Point	11	0	13	**18**	14	0	10	31	0	40
Highborne Cay	6	0	2	9	16	0	**20**	**40**	**38**	0
Lee stocking	0	22	2	9	12	0	7	0	0	**60**
San Salvador	**6**	**11**	**9**	**18**	5	0	7	4	8	0
Long Island	2	11	22	**19**	16	0	5	4	8	0
Georgetown	0	0	2	9	5	0	5	4	8	0
Acklins Island	2	11	7	9	5	**65**	6	4	0	0
French Wells	6	0	5	0	5	14	**20**	13	**38**	0
Total number of specimens	35	8	55	11	58	14	60	23	13	5

* Collection sites are listed starting from north to south.

[1] % Observed frequency relates only to figures down a column.

files were F, G and I. In the Central sites a more uniform distribution between all chemical profiles, with the exception of F, was observed. Noteworthy is also the narrow distribution of chemical profile K, which is found only at Eleuthera point and Lee Stocking Island.

The studied gorgonians were collected from 3 different water depths: the shallow surge zone (0–3m), the intermediate zone (6–10m) and the deep-water zone (20–30m). Seven out of ten gorgonian chemical profiles appeared in all depths, while B, I and K chemical profiles did not inhabit deep water environments (Fig. 2). E and I chemical profiles were the dominant ones at the shallow water zone whereas K and B were found mainly at medium depths.

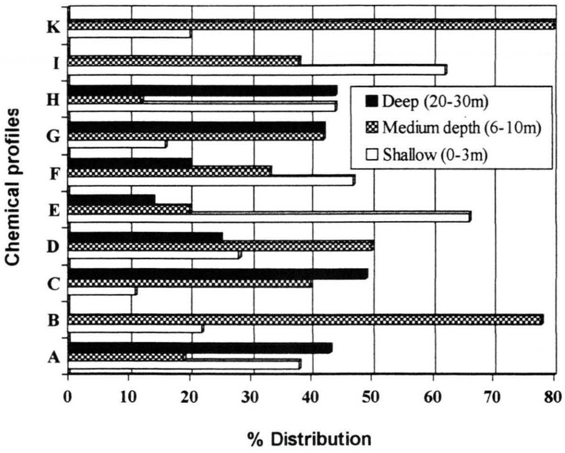


Fig. 2. Depth variation of the studied gorgonian soft corals.

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